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^{13}C-METHACETIN BREATH TEST CORRELATES WITH CLINICAL INDICES OF LIVER DISEASE SEVERITY IN PATIENTS WITH PRIMARY BILIARY CIRRHOSIS

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This prospective study intended to ascertain if cytochrome P450 dependent liver function is affected in early and late histological stages of primary biliary cirrhosis (PBC). The study included 32 female PBC patients (mean age 55.4 years, range 33–70) and 16 aged-matched healthy women (mean age 52.6 years, range 38–65). In every subject a ^{13}C-methacetin breath test (^{13}C-MBT) was applied, and the results were related to histological Ludwig's staging system and several indices of liver disease severity comprising the MAYO-1, MAYO-2, MELD, and Child-Pugh score. The ^{13}C-MBT differentiated healthy controls from the patients with Ludwig IV and Ludwig III histopathological stages of PBC. The most significant relationships (*i.e.* explaining >50% of the variance) were found between measurements of the momentary breath ^{13}C elimination from 6 to 18 minutes as well as the 15-min or 30-min cumulative elimination and the MAYO-1 or MAYO-2 scores. The breath test poorly correlated with histopathological features of PBC, however, it accurately discriminated cirrhotic from non-cirrhotic patients (momentary breath ^{13}C elimination at 40 min, AUROC 0,958). In conclusion, ^{13}C-MBT correlates with clinical scoring systems, especially those specifically designed for PBC (Mayo model) and accurately recognizes the disease at the stage of cirrhosis up to 40 minutes of the test duration.

Key words: *^{13}C-methacetin breath test, cytochrome P450, liver function, primary biliary cirrhosis, carbon dioxide, fibrosis*

INTRODUCTION

Primary biliary cirrhosis (PBC) is an insidious autoimmune disease of the liver that begins in the interlobular and proximal-septal bile ducts. In the next step the inflammation spreads to the entire portal field, sometimes extending to the lobular parenchyma. Histologically, PBC leads to destruction of the bile ducts ("vanishing bile duct syndrome") and ends up in cirrhosis of the liver (1, 2). Median survival time since PBC has been diagnosed is 9.6 years in asymptomatic and 8 years in symptomatic patients, however, the natural history of the disease varies greatly among individual patients. Some patients live long without any symptoms, and a presymptomatic phase may last up to 20 years. Other patients present jaundice and develop hepatic failure in early phases of the disease. A preterminal or accelerated phase with marked jaundice last up to 2 years (3-5).

Based on clinical observations, there are three types of clinical evolution in PBC, namely minimal to slow progression, rapid progression to jaundice and hepatic failure, and progression to portal hypertension without developing deep jaundice (3). There are no identifiable features that distinguish the asymptomatic population who will remain symptom-free from those patients who will develop symptoms, and routine laboratory parameters provide only circumstantial evidence as to the degree of hepatic injury.

Hepatic synthetic function tests such as serum albumin level and prothrombin time are commonly preserved throughout greater part of natural history of PBC and serum alkaline phosphatase levels do not correlate with the severity of bile duct damage (6, 7). Liver biopsy is a standard diagnostic tool in assesment of PBC severity, however, the character of this examination precludes its use from monitoring of disease progression.

The Mayo model is a mathematical equation based on Cox's proportional hazards regression, using six independent variables affecting survival (age, serum bilirubin, serum albumin, prothrombin time, peripheral edema, diuretic treatment). This model was originally developed to estimate the risk of death at the time of PBC diagnosis (1, 2). At present, Mayo model is used to determine the appropriate moment for liver transplantation in patients treated with ursodeoxycholic acid, however, the model is not widely accepted due to low predictability of posttransplant survival (8). A breakthrough in testing liver function was development of methods based on the supply of substrates metabolized exclusively by hepatocytes, while one of the products of the reaction is carbon dioxide (CO_2). Replacement of naturally occurring carbon ^{12}C with radioactive ^{14}C or nonradioactive ^{13}C in the functional group allowed quantitative measurement of labeled CO_2 in the expired air. ^{13}C detection using infrared spectrometry instead of mass spectrometry has considerably reduced the costs of breath tests. Methacetin, a derivative of phenacetin, is the substance highly extracted from the blood, which undergoes O-demethylation in the hepatic microsomal enzyme system (cytochrome P450 1A2) (9). The

close relationship between results of ^{13}C-methacetin breath test (^{13}C-MBT) and liver functional mass was confirmed in animal studies (10).

PBC patients need a non-invasive bedside test to make decisions about response to ursodeoxycholic acid, need for liver transplantation, feasiblity of liver and non-liver surgery, or eligibility for transjugular intrahepatic portosystemic shunt. It was demonstrated that ^{13}C-MBT is accurate in predicting the severity of viral or alcoholic liver cirrhosis showing satisfactory correlation with the Child-Pugh score (11-13). Much less is known about quantification of liver function by ^{13}C-MBT in chronic cholestatic diseases. The aim of this prospective study was to find out whether cytochrome P450 dependent liver function is impaired in different stages of PBC and if results of the ^{13}C-MBT are related with clinical and histopathological estimates of disease severity.

MATERIAL AND METHODS

Thirty two women with PBC were examined (average age 55.4 years, range 33–70). The diagnosis of PBC was based on increased serum alkaline phosphatase level (exceeding by more than 1.5 the upper normal limit), presence of antimitochondrial antibodies (AMA; titer >1:40 in direct immunofluorescence test) and histological features of PBC in liver biopsy specimen. Extrahepatic cholestasis was excluded by abdominal ultrasound, and if necessary by magnetic resonance cholangiopancreatography. None of the patients had a history of chronic viral hepatitis and serum HBsAg and anti-HCV antibodies were undetectable as confirmed by a second generation immunoassay. No one of the patients did systematically use significant amounts of alcoholic beverages. At the time of the study ursodeoxycholic acid, 13–15 mg/kg body mass/day was administered. The patients did not take any drugs known to affect the CYP1A2 activity. The exclusion criteria were liver biopsy performed more than 12 months prior to enrollment (except for patients with decompensated cirrhosis), chronic or acute lung disease, heart failure, unstable cardiac ischemic disease, cancer or any concurrent viral or bacterial infection.

In every patient a set of laboratory parameters were examined which comprised, among others, those necessary to compute several disease severity indices: serum bilirubin, serum creatinin, serum albumin, prothrombin time and international normalized ratio (INR). The following liver disease severity indices were calculated: the Mayo Natural History Model For Primary Biliary Cirrhosis (MAYO-1) (14, 15), the Updated Natural History Model For Primary Biliary Cirrhosis (MAYO-2) (16, 17), the Model of End-Stage Liver Disease (MELD) (18) and the Child-Pugh score (CP) (19). The individual results of those indices are assembled in *Table 1*. The liver biopsy specimens of the PBC patients were histologically classified according to the Ludwig's criteria: stage I – florid inflammatory reaction of the bile ducts; stage II – vanished bile ducts, portal and periportal inflammation and ductular proliferation; stage III – portal fibrosis, typically without bile ducts, bridging fibrosis; stage IV – cirrhosis (20). Cirrhosis was diagnosed in 8 patients. Individual data on liver histology are given in *Table 1*.

A reference group comprised 16 healthy women matched for age with the PBC patients (average age 52.6 years, range 38–65 years). The volunteers declared themselves as being in full health according to the World Health Organization definition (21), which was verified by history questioning and routine physical examination. Additional exclusion criteria from the study comprised symptoms suggestive of impaired absorptive function of the gut, history of surgery affecting the digestive tract anatomy apart from appendectomy. In every subject the normal structure and size of the liver was confirmed by means of an ultrasonographic examination.

The study was conducted in accordance with the Helsinki Declaration, and every volunteer gave a written consent to participate after getting information as to the aim, protocol design and methodology of the study.

Performance of the ^{13}C-methacetin breath test

During an introductory interview the subjects were instructed not to eat any food of naturally increased ^{13}C content, such as products made of maize, cane sugar, pineapple and kiwi fruit for 48 hours preceding the examination (22).

The subjects were examined in the morning, after a 12-h overnight fast. After a 15-min rest in a sitting position, necessary for stabilization of the metabolism, two basal samples of the exhaled air were collected. At the time point designated "0" the subjects took orally 75 mg ^{13}C-methacetin (code INC590P, Euriso-Top S.A., Saint-Aubin, France) dissolved in 200 ml unsweetened black tea. Samples of expiratory air for $^{13}CO_2$ measurement were collected at 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 40, 50, 60, 75, 90, 105, 120, 150, and 180 min after intake of the substrate. The procedure of collecting the breath air was standardized: the subjects took in breath and held it for 10 seconds, then steadily blew the air into a special aluminium covered plastic bag of 1.1 l capacity (Fischer Analysen Instrumente GmbH, Leipzig, Germany) equipped with a mouthpiece and an unidirectional valve (23, 24). The bag was closed with a plastic cork immediately at the end of the exhalation and stored for analysis. Concentrations of $^{13}CO_2$ in the probes of the exhaled air were measured with the use of a non-dispersive isotope-selective infrared spectrometer (*IRIS*, manufactured by Wagner Analysen Technik Vertriebs GmbH, Germany; a model equipped with 16 ports for simultaneous mounting of bags with air samples was used).

The determined $^{13}CO_2$ content within the total pool of the exhaled CO_2 was expressed in δ‰ PDB units, *i.e.* relative to the international standard - the calcium carbonate of the fossil Belemnitella of the cretaceous Pee Dee formation in South Carolina, USA (zero δ‰ PDB corresponds to 1.11123% ^{13}C atoms within $CaCO_3$). The net changes in the $^{13}CO_2$ concentration were conveyed as DOB units (delta over baseline; ‰) according to the formula:

$$DOB_i = \delta‰\ PDB_i - \delta‰\ PDB_0,$$

where i stands for the sample number, and 0 index pertains to the basal sample of the expiratory air. The momentary ^{13}C elimination at time i, D%_^{13}C, expressed in percent administered dose per hour (%/h), was computed according to the equation:

$$D\%_i\ ^{13}C = 100\ (DOB_i/1000\ 0.0112372\ TCO_2)\ /dose$$

where TCO_2 = total expiratory CO_2 production in mmol/h and dose = amount in mmol of ^{13}C-methacetin given to the subject; the TCO_2 was derived by multiplication of the rate 300 mmol/m^2/h by the body surface area computed according to the formula by Haycock *et al.* (25).

From the obtained curve two parameters were derived: the maximum momentary ^{13}C recovery (D_{max}), and the time to its occurrence (T_{max}).

Moreover, the fractional ^{13}C elimination within a given period, C_i_^{13}C, expressed in %, was computed following the trapezoid rule:

$$C_i\ ^{13}C = 0.5\ (DOB_{i-1} + DOB_i)/1000\ \Delta t\ 0.0112372\ TCO_2\ /dose$$

where Δt = the time span between consecutive DOB values.

Ultimately, AUC_i – the cumulative ^{13}C elimination was derived from a stepwise summation of the C_i_^{13}C within the time domain of 0 to 180 min.

Table 1. Individual patients' liver disease severity indices and histopathological staging of primary biliary cirrhosis (PBC).

Patient		Diseases severity scores				Histopathology
No	Age (y)	MAYO-1	MAYO-2	MELD	Child-Pugh	Ludwig's score
1	56	4.75	5.30	7.87	5	2
2	52	4.30	4.79	7.21	5	2
3	55	4.36	4.80	6.43	5	1
4	62	6.31	7.42	10.05	7	4
5	64	6.48	7.67	10.49	7	4
6	59	4.58	5.18	7.42	5	2
7	66	4.88	5.47	7.19	5	3
8	53	4.82	5.23	6.43	5	2
9	57	4.34	4.77	6.81	5	2
10	56	5.42	6.20	9.28	5	3
11	38	4.30	4.82	9.31	6	3
12	53	3.78	4.01	6.43	5	2
13	63	8.60	10.3	24.3	10	4
14	61	4.31	4.74	6.43	5	4
15	48	4.54	5.05	7.72	5	3
16	61	4.31	4.72	8.68	5	1
17	67	4.17	4.54	6.43	5	3
18	50	3.69	3.96	6.43	5	2
19	67	9.41	11.2	17.5	11	4
20	53	3.57	3.78	6.43	5	1
21	49	7.09	8.54	15.7	8	3
22	36	3.81	4.06	7.19	5	2
23	39	4.45	4.99	8.16	5	3
24	70	6.58	7.78	10.4	7	4
25	69	5.02	5.71	6.73	5	3
26	52	6.59	7.34	15.7	7	4
27	59	4.22	4.67	6.43	5	1
28	59	3.83	4.09	6.43	5	1
29	33	2.80	2.76	6.43	5	1
30	59	4.58	5.07	6.43	5	3
31	48	3.27	3.40	6.43	5	2
32	59	6.50	7.74	12.8	8	4

A recent reproducibility study revealed that with a within-subject study protocol involving paired examinations taken at a median interval of 18 days, a sample of twelve subjects offers the smallest detectable difference in D_{max}, T_{max}, and AUC amounting to: 3.51 %/h, 3.8 min, and 1.26%, respectively (at p=0.05 level, two-tailed).

Statistical analysis

The results obtained were subjected to analysis of variance (ANOVA). In the case sets of parameters changing within a time domain has to be compared, such as curves of the momentary or cumulative ^{13}C breath elimination, the analysis involved the main effect of health/disease status and its interaction with time. Otherwise a main effect of health/disease status was tested. In case ANOVA disclosed a significant effect, subsequently significance of differences between means was checked *post hoc* with the Tukey's honest significant difference (HSD) test. Relationships between variables were checked with the Spearman rank correlation because this approach does not require any assumption with regard to linearity of the connection between variables (26).

The diagnostic performance of selected parameters of ^{13}C-MBT was determined by constructing receiver operating characteristic (ROC) curves and calculating the area under ROC (AUROC). Statistical significance was set at the $p<0.05$ level, two-tailed. Results are presented as averages ± standard error of the mean (S.E.). All statistical analyses were performed with the use of Statistica 8.0 software, licence # JGNP711A903815AR-X.

RESULTS

Kinetic parameters of ^{13}C-methacetin breath test

After a peroral intake of 75 mg ^{13}C-methacetin the curves reflecting the momentary ^{13}C elimination within the exhaled air exhibited a typical biphasic time course - a rapid rise switched next to a less steep decline. ANOVA revealed that between 6 and 50 min in patients with Ludwig IV histological stage, and between 12 and 18 min in patients with Ludwig III stage the momentary breath ^{13}C elimination was statistically significantly lower than in healthy controls (*Fig. 1*). ANOVA disclosed a

Table 2. Kinetics of breath ^{13}C elimination in female primary biliary cirrhosis (PBC) patients grouped according to the histological Ludwig's staging system (LSS) and in age-matched healthy women.

	Healthy, N=16	Histological Ludwig's stage			
		LSS I, N=6	**LSS II,** N=9	**LSS III,** N=9	**LSS IV,** N=8
D_{max} (%)	**34.6** *±1.9*	**34.1** *±3.2*	**31.0** *±3.0*	**28.4** *±3.4*	**15.1** [a,b,c,d] *±3.1*
T_{max} (min)	**18.9** *±1.3*	**20.0** *±2.1*	**20.7** *±1.5*	**23.3** *±1.8*	**30.0** [e] *±4.6*

Parameters of the breath test: D_{max} = maximum momentary ^{13}C recovery, T_{max} = time of D_{max} occurrence.
Differences statistically significant: [a] p=0.00017 *vs*. healthy, [b] p=0.0016 *vs*. LSS I, [c] p=0.0036 *vs*. LSS II, [d] p=0.020 *vs*. LSS III, [e] p=0.0066 *vs*. healthy.

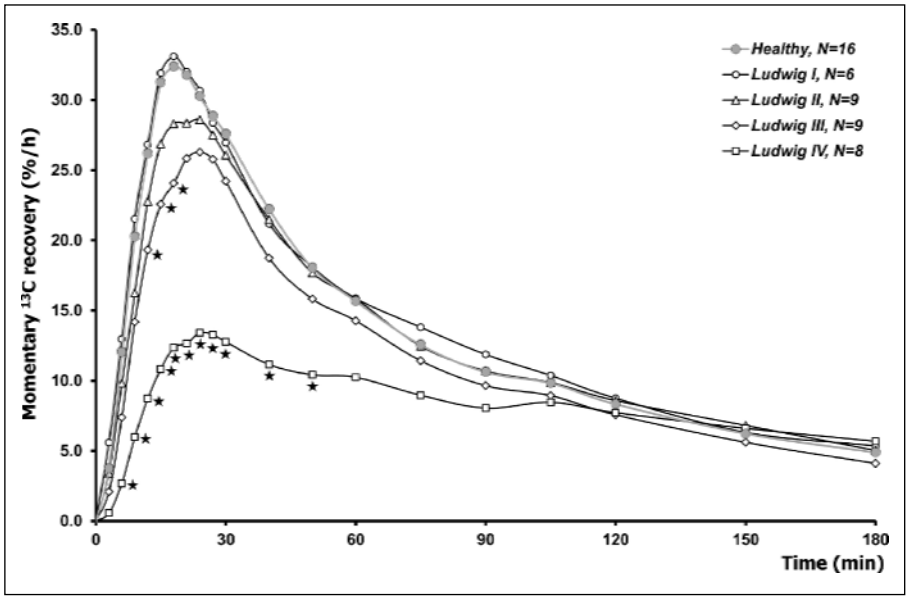

Fig. 1. Breath ^{13}C elimination in female PBC patients grouped according to the histological Ludwig's staging system and age-matched healthy women; asterisks indicate values statistically different from healthy controls; for legibility S.E.M. bars are omitted.

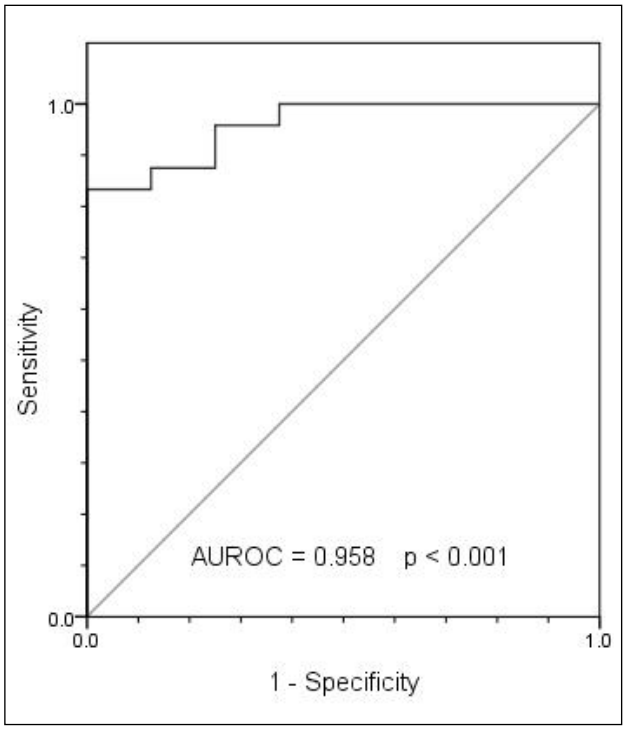

Fig. 2. ROC curve of the momentary ^{13}C breath elimination at 40 min for discrimination of cirrhosis against non-cirrhotic PBC. The diagonal line represents that achieved by chance alone (area under the curve 0.50); the ideal area under the curve is 1.00.

statistically significant main effect of the subject status (5 levels: healthy, Ludwig I, II, III, IV) upon the kinetics of breath ^{13}C elimination. Results of the subsequent *post hoc* comparisons are assembled in *Table 2*. It can be inferred from that the D_{max} was significantly lower in patients with Ludwig IV stage than either in healthy controls or any other subgroup of PBC patients. The patients with Ludwig IV stage had also the longest T_{max}, but this parameter distinguished them statistically significantly from healthy subjects only. Finally, according to ANOVA the cumulative breath ^{13}C elimination between 21 and 180 min in patients with Ludwig IV histological stage, and between 60 and 180 min in patients with Ludwig III stage was statistically significantly lower than in healthy controls (*Table 3*).

Relationship between the results of ^{13}C-methacetin breath test and the indices of liver disease severity

Spearman correlation coefficients were computed between a set of the ^{13}C-MBT parameters taken as dependent variables and the MAYO-1, MAYO-2, MELD and Child-Pugh scores, as well as stages of Ludwig's PBC classification (independent variables). The results of this analysis are assembled in *Table 4*. Although all the correlation coefficients were statistically significant, the strongest relationship existed between the breath ^{13}C elimination from the 6 to 18 minute or the AUC_{15} or AUC_{30} and the MAYO-1 or MAYO-2 scores.

Patients with cirrhosis and without cirrhosis differed significantly ($p<0.02$) with respect to D_{max} 13.0%/h (range 8.18–20.8) *vs*. 29.9%/h (range 24.9–37.3) and T_{max} 28.5 min (range 24.0–45.0) *vs*. 21.0 min (18.0–25.5), respectively. The cumulative ^{13}C recovery at 30 min after administration of ^{13}C-

Table 3. Cumulative breath ^{13}C elimination in female primary biliary cirrhosis (PBC) patients grouped according to the histological Ludwig's staging system (LSS) and in age-matched healthy women.

	Healthy, N=16	Histological Ludwig's stage			
		LSS I, N=6	**LSS II,** N=9	**LSS III,** N=9	**LSS IV,** N=8
AUC_3	**0.1** ±*0.0*	**0.1** ±*0.0*	**0.1** ±*0.0*	**0.1** ±*0.0*	**0.0** ±*0.0*
AUC_6	**0.5** ±*0.1*	**0.6** ±*0.1*	**0.4** ±*0.0*	**0.3** ±*0.1*	**0.1** ±*0.0*
AUC_9	**1.3** ±*0.1*	**1.5** ±*0.3*	**1.1** ±*0.1*	**0.8** ±*0.2*	**0.3** ±*0.1*
AUC_{12}	**2.5** ±*0.2*	**2.7** ±*0.4*	**2.0** ±*0.2*	**1.7** ±*0.4*	**0.7** ±*0.3*
AUC_{15}	**3.9** ±*0.3*	**4.1** ±*0.6*	**3.3** ±*0.3*	**2.7** ±*0.5*	**1.2** ±*0.4*
AUC_{18}	**5.5** ±*0.3*	**5.8** ±*0.8*	**4.7** ±*0.5*	**3.9** ±*0.7*	**1.7** ±*0.6*
AUC_{21}	**7.1** ±*0.4*	**7.4** ±*0.9*	**6.1** ±*0.6*	**5.1** ±*0.8*	**2.4**★ ±*0.7*
AUC_{24}	**8.7** ±*0.5*	**9.0** ±*1.0*	**7.5** ±*0.7*	**6.4** ±*0.9*	**3.0**★ ±*0.9*
AUC_{27}	**10.1** ±*0.6*	**10.4** ±*1.2*	**8.9** ±*0.8*	**7.7** ±*1.0*	**3.7**★ ±*1.0*
AUC_{30}	**11.6** ±*0.6*	**11.8** ±*1.2*	**10.2** ±*0.9*	**9.0** ±*1.1*	**4.3**★ ±*1.1*
AUC_{40}	**15.7** ±*0.8*	**15.8** ±*1.5*	**14.2** ±*1.1*	**12.6** ±*1.4*	**6.3**★ ±*1.4*
AUC_{50}	**19.1** ±*0.9*	**19.1** ±*1.6*	**17.5** ±*1.2*	**15.4** ±*1.5*	**8.1**★ ±*1.7*
AUC_{60}	**21.9** ±*1.0*	**21.9** ±*1.7*	**20.3** ±*1.3*	**17.9**★ ±*1.7*	**9.9**★ ±*1.9*
AUC_{75}	**25.4** ±*1.0*	**25.6** ±*1.8*	**23.8** ±*1.4*	**21.2**★ ±*1.8*	**12.3**★ ±*2.2*
AUC_{90}	**28.3** ±*1.1*	**28.8** ±*1.8*	**26.7** ±*1.4*	**23.8**★ ±*2.0*	**14.4** ±*2.4*
AUC_{105}	**30.9** ±*1.2*	**31.6** ±*1.8*	**29.3** ±*1.5*	**26.1**★ ±*2.1*	**16.4** ±*2.6*
AUC_{120}	**33.2** ±*1.3*	**34.0** ±*1.9*	**31.6** ±*1.5*	**28.2**★ ±*2.2*	**18.5**★ ±*2.8*
AUC_{150}	**36.9** ±*1.4*	**37.8** ±*1.9*	**35.4** ±*1.4*	**31.5**★ ±*2.3*	**22.0**★ ±*3.0*
AUC_{180}	**39.7** ±*1.5*	**40.7** ±*1.9*	**38.4** ±*1.4*	**33.9**★ ±*2.5*	**25.1**★ ±*3.1*

AUC_t = cumulative ^{13}C recovery (t indicates the time span in minutes for which AUC was computed).
Asterisks indicate statistically significant differences *vs*. healthy controls.

Table. 4. Relation between quantitative parameters of the ^{13}C-methacetin breath test and the liver disease severity indices in 32 female primary biliary cirrhosis (PBC) patients.

	$D\%_6$	$D\%_{12}$	$D\%_{18}$	$D\%_{24}$	$D\%_{30}$	$D\%_{40}$	$D\%_{50}$	$D\%_{60}$	D_{max}	T_{max}	AUC_{15}	AUC_{30}	AUC_{60}	AUC_{90}	AUC_{120}	AUC_{180}
MAYO-1	**-0.77**	**-0.74**	**-0.71**	-0.58	-0.56	-0.59	-0.51	-0.48	-0.68	0.57	**-0.75**	**-0.71**	-0.66	-0.64	-0.63	-0.57
MAYO-2	**-0.77**	**-0.75**	**-0.72**	-0.61	-0.58	-0.61	-0.54	-0.50	-0.69	0.57	**-0.76**	**-0.72**	-0.67	-0.65	-0.65	-0.58
MELD	-0.56	-0.60	-0.62	-0.55	-0.50	-0.50	-0.39	-0.35	-0.57	0.54	-0.60	-0.56	-0.53	-0.51	-0.50	-0.44
Child-Pugh	-0.64	-0.62	-0.64	-0.65	-0.65	-0.64	-0.51	-0.47	-0.62	0.48	-0.64	-0.64	-0.63	-0.61	-0.57	-0.52
Ludwig	-0.62	-0.54	-0.59	-0.59	-0.62	-0.64	-0.58	-0.52	-0.54	0.41	-0.58	-0.60	-0.62	-0.63	-0.64	-0.61

$D\%_t$ = momentary ^{13}C recovery in expiratory air at given time point, D_{max} = maximum momentary ^{13}C recovery, T_{max} = time to reach the D_{max}, AUC_t = cumulative ^{13}C recovery calculated as the area under the momentary ^{13}C recovery curve for a given time span.
All the Spearman rank correlation coefficients given in the table are statistically significant; highlighted with a bold case in shaded cells are those correlation coefficient which explain >50% of the variance, *i.e*. the module of which exceeds 0.707.

methacetin significantly differentiated patients with and without cirrhosis 3.75% (range 1.66–6.37) *vs*. 10.2% (range 8.54–11.9); $p<0.001$. In ROC analysis made for momentary ^{13}C recovery significant discriminations between cirrhotic (Ludwig IV) and non-cirrhotic PBC patients were obtained starting from 3 min, with 16.3%/h as the best cut-off value, however, the highest AUROC was calculated for the measurement at 40 min (*Fig. 2*). For all investigated ^{13}C-MBT parameters we found no statistically significant differences between non-cirrhotic PBC patients and healthy controls.

DISCUSSION

It has been shown that grade of inflammation and degree of fibrosis in chronic hepatitis C have significant influence on result of ^{13}C-MBT (27, 28). This test also showed a good performance in detection of liver cirrhosis and posttransplant monitoring of hepatic function (29). There are, however, a few problems preventing ^{13}C-MBT from wide clinical application. Skipping purely technical aspects, the criticism is focused on the dependence of ^{13}C-MBT on hepatic oxygen supply and the presence of known and unknown factors inhibiting or activating cytochromes P450 (31). Among our patients no one had clinical symptoms of hepatopulmonary syndrome or other pulmonary disease. Furthermore, we did much effort to reduce an effect of smoking or ingestion of drugs/alcohol on ^{13}C-MBT.

Majority of studies with ^{13}C-MBT were performed in chronic liver disease induced by HCV infection, however, the pathogenesis of PBC markedly varies from viral liver injury, and different enzymes may be affected by these etiologically dissimilar diseases. Chronic cholestasis is associated with accumulation of cytotoxic bile acids within hepatocytes. Besides, the inflammation and fibrosis in PBC reside over

long time in portal triads, before they ultimately extend into hepatic lobules. Bridging fibrosis associated with loss of hepatocytes seems to be a turning point in the natural history of this disease.

Until now, there was only one study using ^{13}C-MBT in PBC patients (32). This study showed reduced metabolism of ^{13}C-methacetin in 19 non-cirrhotic PBC patients in comparison with healthy controls. Interestingly, restricted liver function was found in 8 patients who had normal serum levels of bilirubin and alkaline phosphatase. Despite high sensitivity of ^{13}C-MBT in detection of cholestasis, the test did not correlate with severity of histological injury assessed by Ludwig's criteria. In contrast to Holtmeier's study (32), in our hands the ^{13}C-MBT was not diagnostically very sensitive, as the test did not differentiate patients with pre-cirrhotic stage of PBC from healthy controls. It should also be noticed that no asymptomatic cases of PBC were enrolled to our study. In patients with PBC the curve ascended more gently and time of peak exhalation of $^{13}CO_2$ was delayed. On the other hand, the descending arm of the curve showed tendency to normalize over time. Significant differences occurred for measurements made between 10 and 25 minutes following ingestion of ^{13}C-methacetin, however, differences in AUC persisted to 60 minutes. In analysis of ROC curves the best discrimination of cirrhotic patients was achieved at 40 min of the breath test. Therefore, taking into account the economical aspect and clinical utility, the ^{13}C-MBT could be significantly simplified, being completed in PBC patients after 40 minutes. In the study by Holtmeier *et al*. (32) the most important differences where also found during initial 30 minutes of the examination.

Our study confirms the lack of close relationship between ^{13}C-MBT and histological staging of liver disease. It is not surprising as histological scores rather summarize very variable pathomorphological features than evaluate liver function. Moreover, it is known that distribution of biliary duct injuries and fibrosis may be heterogenous in PBC and biopsy core is only a small sample of the liver. Stages I and II in Ludwig's classification are focused on the pathology limited to portal triads, while stages III and IV provide information on severity of parenchymal fibrosis. Therefore, the liver function should be preserved before the disease steps forward to stage III. This concept is true under condition that intracellular retention of bile acids has no significant adverse impact on liver cell functions, including activity of microsomal enzymes. In this study the ^{13}C-MBT discriminated between patients with cirrhosis and earlier stages of PBC. This finding is important as many clinical decisions in PBC patients are based on the knowledge of hepatic progressive fibrosis.

In clinical practice, overall assessment of liver disease severity is concluded not only from liver biopsy but also from physical examination and routine laboratory tests. ^{13}C-MBT correlated perfectly with different clinical prognostic scores, both specific (Mayo model) and non-specific (MELD, Child-Pugh) for PBC. These prognostic scores are clinically useful, but sometimes suffer important limitations due to low specificity for liver failure. Such clinical conditions like Gilbert's syndrome, renal injury, cardiac failure, infection or corticosteroid therapy may modify results of these scoring systems (31). In such patients the use of ^{13}C-MBT is justified, because by the fact that only insignificant amounts of P450 are found outside the liver, the test is believed to be specific for this organ.

In conclusion, the use of the ^{13}C-MBT may provide a powerful tool in decision making at the bedside in PBC patients, as this test closely correlates with Mayo model and enables differentiation cirrhosis from non-cirrhotic stages and healthy liver.

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